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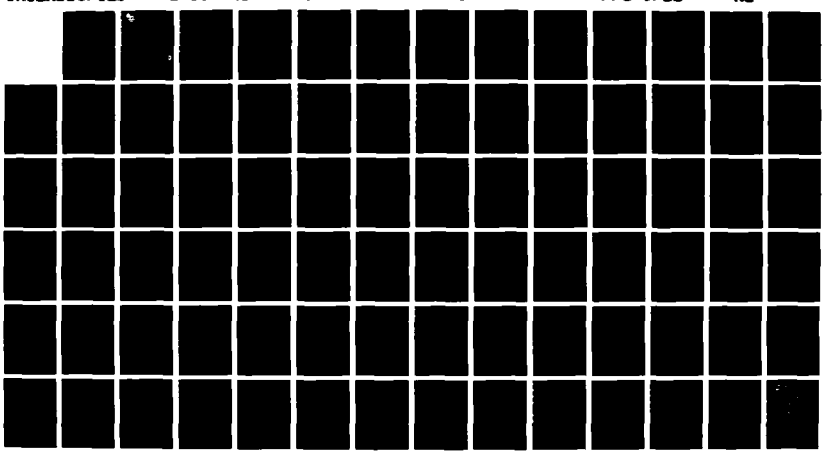

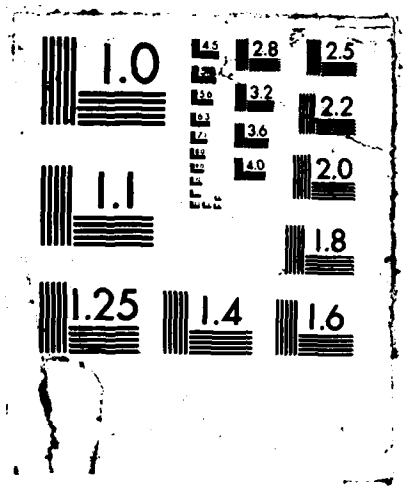
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Institute Report No. 247

Teratogenic Potential of Ethylene Thiourea (ETU), a Positive Control, in Sprague-Dawley Rats

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and
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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

Toxicology Series: 53

OCTOBER 1987

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Teratogenic Potential of Ethylene Thiourea (ETU), a Positive Control, in Sprague-Dawley Rats (Toxicology Series 53) -- Coppes, Hanes, and Korte

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

This material has been reviewed by Letterman Army Institute of Research and there is no objection to its presentation and/or publication. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense. (AR 360-5)

Edwin S. Seature 9 Oct 87

(Signature and date)

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The teratogenic potential of ethylene thiourea (ETU) was tested in pregnant Sprague-Dawley rats. An aqueous solution of ETU was administered by oral gavage at a dose level of 40 mg/kg on Days 6 through 15 of gestation. The control group received a vehicle containing 21.5% Tween 80, 18.5 absolute ethanol, 37.5% 50 mM citrate buffer, and 22.5% distilled water. Fetuses were delivered by cesarean section on Day 20, weighed, examined externally, and either processed in Bouin's solution for visceral examination or alizarin red stain for skeletal examination. ETU produced teratogenic and embryotoxic effects in Sprague-Dawley rats.

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ABSTRACT

The teratogenic potential of ethylene thiourea (ETU) was tested in pregnant Sprague-Dawley rats. An aqueous solution of ETU was administered by oral gavage at a dose level of 40 mg/kg on Days 6 through 15 of gestation. The control group received a vehicle containing 21.5% Tween 80, 18.5 absolute ethanol, 37.5% 50 mM citrate buffer, and 22.5% distilled water. Fetuses were delivered by cesarean section on Day 20, weighed, examined externally, and either processed in Bouin's solution for visceral examination or alizarin red stain for skeletal examination. ETU produced teratogenic and embryotoxic effects in Sprague-Dawley rats.

Key Words: Developmental Toxicity, Teratology, Ethylene thiourea, Rat

PREFACE

TYPE REPORT: Teratogenic GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR: US Army Medical Research and Development Command
Fort Detrick, MD 21701-5010

PROJECT: 3516277A875, Medical Defense Against Chemical Agents
WU 304, APC TL04

GLP STUDY NUMBER: 82021

STUDY DIRECTOR: Don W. Korte, Jr., PhD, MAJ MSC

PRINCIPAL INVESTIGATOR: Martha A. Hanes, DVM, CPT VC

CO-PRINCIPAL INVESTIGATORS: Valerie G. Coppes, BS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, SOPs, and raw data will be retained in the LAIR Archives. Alizarin specimens will be retained in the LAIR Pathology Archives.

TEST SUBSTANCE: Ethylene Thiourea

INCLUSIVE STUDY DATES: 31 August 1982 - 15 March 1983

OBJECTIVE: The purpose of this study was to provide historic data demonstrating the Sprague-Dawley rat is sensitive to a known teratogen in the LAIR teratogenicity test system.

ACKNOWLEDGMENTS

SP5 Thomas P. Kellner, BA; SP5 Paul D. Mauk, BS; SP5 Lawrence Mullen, BS; SP5 Justo Rodriguez, BS; SP4 Evelyn Zimmerman, Carolyn M. Lewis, MS; and Yvonne C. Johnson, BS, assisted in the research; COL John Marshall, PhD, and COL John Fruin, DVM, PhD, gave professional guidance.

SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 82021 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

14 Oct 87

DON W. KORTE, JR., PhD / DATE
MAJ, MSC
Study Director

14 Oct 87

MARTHA A. HANES, DVM / DATE
CPT, VC
Principal Investigator

14 Oct 87

VALERIE G. COPPES, BS / DATE
DAC
Co-Principal Investigator

DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129-6800

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 30 Sep 87

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

1. I hereby certify that in relation to LAIR GLP Study 82021, the following inspections were made.

Insemination Determination	(Phase II)	10 Feb 83
Dosing and Weighing	(Phase I)	19, 29 Oct 82
	(Phase II)	23, 24 Feb 83
Unscheduled Sacrifice	(Phase I)	26 Oct 82
Scheduled Sacrifice	(Phase I)	2 Nov 82
	(Phase II)	3 Mar 83
Fetal Visceral Observations	(Phase II)	21 Apr 83
Fetal Skeletal Observations	(Phase II)	5 Aug 84

2. The report entitled "Teratologic Potential of Ethylene Thiourea (ETU), a Positive Control, in Sprague-Dawley Rats," Toxicology Series 53, and the raw data were audited on 11 Jun 87, 24 Aug 87, 15 Sep 87, and 18 Sep 87.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

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Teratogenic Potential of Ethylene Thiourea (ETU), a Positive Control, in Sprague-Dawley Rats--Coppes et al

The Toxicology Group, LAIR, has been tasked to perform toxicological evaluation of compounds to include teratogenic testing. Regulatory agencies require a concurrent positive control group unless historic data from the laboratory performing the teratogenicity test demonstrate that the strain of animals being used is sensitive to known teratogenic agents (1). This report presents the results of a concurrent positive control group, ethylene thiourea (ETU), from LAIR GLP Study 82021 (2).

Objective of the Study

The purpose of this study was to provide historic data demonstrating the Sprague-Dawley rat is sensitive to a known teratogen in the LAIR teratogenicity test system.

MATERIALS

Rationale for Selection of the Positive Control

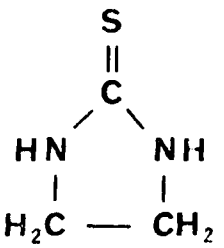
Ethylene thiourea, a decomposition product of the ethylenebisdithiocarbamate fungicides, is a known teratogen. Malformations induced by ETU are due to an extensive cell necrosis which occurs in the affected organs at an early stage of embryonic development (3,4). Preliminary studies in our laboratory showed ETU was a consistent teratogen in Sprague-Dawley rats.

Positive Control Substance

Chemical Name: 2-Imidazolidinethione, Ethylene Thiourea

Chemical Abstract Service Registry No.: 96-45-7

Molecular Structure: $C_3H_6N_2S$



Molecular Weight: 102.17

Source: Eastman Kodak Company
Rochester, NY 14650

Lot No: A7A Cat No P5950

Stability: Stable at elevated temperature over wide pH range (3).

Published Toxicity Data: ETU is a known carcinogen, mutagen, and teratogen (3).

Vehicle

The vehicle for ETU was distilled water.

Rationale for Selection of the Negative Control

The positive control groups were run concurrent with the test substance 4-nitrophenyl methyl phenyl phosphinate (MPP), GLP Study 82021. A vehicle containing 21.5% polysorbate 80 (Tween 80), 18.5% absolute ethanol, 37.5% 50 mM citrate buffer (pH 3.2), and 22.5% distilled water was selected to solubilize and stabilize the MPP (2). Since a distilled water control group was not run, the vehicle control group for MPP is shown in this report as a negative control for comparison with the positive control.

Additional information about the negative control appears in Appendix A.

Animal Data

Young adult Sprague-Dawley rats were obtained from Bantin-Kingman, Fremont, CA. Due to its size, the study was conducted in two phases. Phase I required 85 female and 41 male rats and Phase II required 103 female and 52 male rats (including those rats needed for the MPP dose

groups). Two females from Phase I and 6 females from Phase II were randomly selected for quality control necropsy. Animals were identified by sequentially numbered metal eartags. The weight ranges of rats were as follows:

Phase	Receipt		Start of Breeding	
	Females (gm)	Males (gm)	Females (gm)	Males (gm)
I	250-296	249-286	261-331	406-504
II	193-308	223-299	240-327	391-589

Historic data on sporadic malformations in rats are well-documented (5).

Husbandry

Upon arrival at LAIR, rats were housed individually in wire mesh rack cages with automatic water dispensers for the quarantine period. Animals were fed Purina Certified Rodent Chow 5002 (Ralston Purina Company, St Louis, MO) and tap water ad libitum throughout the study. No contaminants or naturally occurring substances were expected to influence the study. During breeding 1 male and 2 females were placed in polycarbonate cages with hardwood chip bedding, wire lids, and water bottles. After breeding the males were returned to the wire mesh rack cages; the females remained in the polycarbonate cages (2 females of the same dose group and breeding date per cage).

In Phase I, room temperature ranged between 64 and 74°F (17.8 and 23.3°C) and relative humidity between 58 and 77%. In Phase II, temperature ranged between 68 and 74°F (20.0 and 23.3°C) and relative humidity ranged between 47 and 70%. Several times during Phase II the relative humidity spiked to 90% for a short period of time. The fluctuation of relative humidity is not expected to have an impact on the outcome of the study. The photoperiod was 13 hours of light per day (0630-1930 hours).

METHODS

Methods used are described in detail in LAIR OP-STX-40 "Teratology Testing Procedure" (6) and were in accordance with Environmental Protection Agency test standards for teratogenic health effects (1).

Acclimation

Females were acclimatized before start of breeding for 8 and 18 days for Phases I and II, respectively. Males were acclimatized for at least 6 weeks.

Group Assignment

Females were assigned to groups according to LAIR OP-ISG-21 "Animal Randomization Procedure" (7) on the Data General ECLIPSE C/330 computer. Twenty random sequences of numbers corresponding to the number of dose groups were generated. When females became sperm positive, they were assigned to the dose group. Animals were selected for quality control necropsy according to a random number table.

Dose Administration

Sperm-positive females were dosed daily between 0800 - 1200 hours from Day 6 through Day 15 of gestation by oral intubation using an 18-gauge, 3-inch gastric gavage needle (Popper and Sons, Inc, New Hyde Park, NY 11040). Dosing was conducted without sedation or anesthesia of the animals. Positive control animals received 40 mg/kg/day ETU. The volume of dosing solution for each female was based on the Day 6 (Day 0 was the day sperm were detected in vagina) body weight and that dose was used throughout the dosing period. Doses were calculated by a program on a Hewlett-Packard 98A calculator. The negative control was given at a constant volume of 1 ml per animal. Phase I females were dosed from 19 Oct 82 through 30 Oct 82. Phase II females were dosed from 14 Feb 83 through 27 Feb 83.

Compound Preparation

A quantity of ETU solution sufficient for the entire dosing period of each phase was prepared. For Phase I a 20 mg/ml solution and for Phase II a 40 mg/ml solution of ETU in distilled water (w/v) were prepared. The solutions were heated to approximately 40°C to attain complete dissolution, aliquoted into vials for each day of dosing to prevent contamination, and stored at room temperature. For each day of dosing, 1 vial was warmed to approximately 40°C to redissolve crystallized ETU. No analysis was performed on the ETU solutions.

The negative control solution was prepared at the beginning of each phase, aliquoted into vials for each day of dosing, and refrigerated. Before daily dosing, a vial of negative control solution was placed in a beaker of hot tap water to bring the solution to room temperature.

Breeding

After the quarantine period, each male was placed in the breeding cage with 2 females. Females were checked each morning for evidence of insemination. Day 0 for each female was the day sperm were observed in her vaginal smear. Sperm-positive females were assigned to dose groups as previously described. Sperm-positive females were separated from the males and placed with another female of same breeding date and dose group.

The breeding period was 3 days for Phase I and 5 days for Phase II. The number of positive females was limited to approximately 30 per day. Matings were terminated when an adequate number of females were sperm positive. Those females which were not sperm positive at the completion of the breeding period were removed from the study.

Cesarean Section Procedure

Dams were weighed and euthanized with CO_2 gas on Day 20 of gestation. Non-pregnant females were examined, and removed from the study. Gravid uteri were examined for number and placement of implantation sites, resorptions, and live and dead fetuses. Corpora lutea were not counted. The uterus and ovaries were removed, the dam examined for gross visceral signs of toxicity and reweighed. Fetuses were assigned alternately to either skeletal or visceral examination. Each fetus was sexed, weighed, and examined externally.

Fetuses assigned for skeletal examination were placed in 70% ethanol for several hours and eviscerated. They were then processed by the alizarin red S staining technique of Crary (8). After processing, the specimens were stored in glycerol with a few crystals of thymol to inhibit bacterial and mold growth. Fetuses assigned for visceral examination were placed in Bouin's solution. The body walls were pierced to allow penetration of the fixing solution.

Observations and Records

Pregnant females were weighed on Day 0 and every other day through Day 20. Females were observed daily from Day 0 through Day 20 for clinical signs of toxicity, abortion, or premature delivery. Clinical signs were not recorded for Phase I animals except at cesarean section. Date, time, and amount of dosing solution administered were recorded during the daily dosing on Days 6 through 15. At cesarean section, uterine data and body weight, and results from gross examination of the dam were recorded. The gravid maternal weight was termed the "Actual Day 20" weight. The maternal weight after removal of the uterus and fetuses was termed the "Corrected Day 20" weight.

Fetal weight, sex, and external examination findings from live fetuses were recorded. Bouin's fetuses were examined under low magnification by the modified Wilson freehand razor blade sectioning technique (9). The alizarin skeletons were examined under low magnification on a light box for degree of ossification, malformations, and alignment. The number of ossified sternebrae, ribs, caudal vertebrae, metacarpals, and metatarsals were counted.

Schedule of Study Events

The study was divided into 2 phases to allow adequate time for animal care, fetal processing, and fetal examination. Upon arrival several animals from each phase were sent for quality control necropsy. The historical listing of study events is given in Appendix B.

Statistical Analysis

The data were analyzed with BMDP software on a Data General ECLIPSE C/330 computer (10). Methods used are described by Hollander and Wolfe (11). Data from both phases were combined for analysis. The litter or litter mean was used as the experimental unit. All tests were run at the .05 level of significance. In this report the term "significant" indicates a statistically significant difference.

The maternal body weights, weight changes (Corrected Day 20 - Day 0), and fetal weights of the positive control were compared with the negative control group by the Student's t-test. The fetal examination findings of the positive control group were compared with the findings from the negative control group by the Fisher's Exact test. The number of implantations, percent resorptions, percent live fetuses, percent male fetuses, and ossification data of the positive control group were compared with the negative control group by the Mann-Whitney test.

Deviations from Original Protocol

Animals were dosed from Day 6 through Day 15, the period of major organogenesis in the rat. The original protocol specified that the positive control animals be dosed once and the vehicle control animals be dosed from Day 6 through Day 17. The dose was calculated on the Day 6 body weight rather than Day 0 as stated in the protocol. Females were weighed every other day instead of every day. Pregnant females were housed 2 per cage rather than gang caged.

These deviations from the protocol did not affect the outcome of the study and actually brought the study into conformation with regulatory guidelines (1).

Animals Excluded from Study

Due to inadvertent destruction of Bouin's processed fetuses, 2 positive control females, 82D00713 and 82D00738, and 1 negative control female, 82D00759, were eliminated from Phase I. Data from the skeletal-processed fetuses from these females were not included in this report. However, these data will be archived with other raw data from GLP Study 82021.

Two negative control animals were misdosed, 82D00716 on 26 Oct 82 and 83D00033 on 16 Feb 83, and subsequently they were removed from the study. Misdosing was confirmed by necropsy findings.

Raw Data and Final Report Storage

A copy of the final report, study protocol, addenda, raw data, and SOPs will be retained in the LAIR Archives. Alizarin specimens will be retained in LAIR Pathology Archives.

RESULTS

Quality Control Necropsy

Tissues from Phase I quality control rats were normal by microscopic examination. Microscopic examination of Phase II tissues revealed sinusitis in all 6 females. Since all other tissues were normal and weight gain of the females was normal, the remaining females from that shipment were used for Phase II. The sinusitis appeared to have no effect on the results of the study.

Maternal Data

The individual maternal weights are listed in Appendix C and the group mean maternal weights are presented in Table 1. There were no significant differences in the weights or weight gains (Corrected Day 20 - Day 0) between the two groups.

There were no maternal deaths in either of the dose groups. Individual maternal clinical signs are listed in Appendix D. Cumulative clinical signs per group during the pretreatment (Day 0 through Day 5), treatment (Day 6 through Day 15), and posttreatment (Day 16 through Day 20) periods are found in Table 2. There were few clinical signs of toxicity in either group. Generally these signs were seen in only 1 animal per group, occurred randomly throughout the dosing period, and lasted 1 or 2 days.

The number of animals assigned to each group, number of animals that died during the study, and number and percent of animals that were

pregnant are presented in Table 3. Pregnancy rate was 68% in the negative control group and 96% in the positive control dose group.

Cesarean/Fetal Data

The individual number of implantations, resorptions, percent resorptions, and number and percent of fetuses dead and live are listed in Appendix E. The number, sex, and mean fetal weight per litter are found in Appendix F. These data are summarized in Table 4. There were no differences in the number of implantations, resorptions, and live and dead fetuses between the groups. The mean fetal weight and mean male fetal weight of the positive control group were significantly lower than the negative control group. The mean female weights were also lower in the positive control group but they were not significant at the 0.05 level. The positive control group had a significantly lower percentage of male fetuses than the vehicle control group.

Each fetus was examined for variations, retarded development, and anomalies both externally during the cesarean section delivery and again after visceral or skeletal processing. Descriptions of the examination findings were recorded. These descriptions with their corresponding incidences are listed in Appendices G, H, and I for the external, visceral, and skeletal examinations. Summaries for incidence of each anomaly and variant appear in Tables 5, 6, and 7. Fetuses with multiple anomalies and variants are listed in more than one descriptive category but are counted only once in the totals. Appendix J lists the number of fetuses per litter with anomalies and variants in the external, visceral, and skeletal examination findings. Appendix K shows the number of fetuses per litter with any anomalies and variants. Table 8 shows the group summary of the number of fetuses and the number of litters containing fetuses with anomalies and variants for the external, visceral, and skeletal examinations and a summary of number of fetuses and litters containing fetuses with any anomalies and variants. The positive control group had significantly increased numbers of fetuses with anomalies and variants and significantly increased numbers of litters containing fetuses with anomalies and variants for the external, visceral, skeletal, and combined examination findings than the negative control group.

The litter mean numbers of sternebrae, caudal vertebrae, metacarpals, and metatarsals ossified are presented in Appendix L and the summary by group is presented in Table 9. Comparing the positive control group with the negative control group, there was no difference in the number of sternebrae, but there were significantly fewer caudal vertebrae, metacarpals, and metatarsals ossified in the positive control group.

Table 1

Maternal Body Weights* and Weight Changes†

Day	Positive Control	Negative Control
0	281.0 ± 14.5	277.8 ± 22.1
6	315.1 ± 16.8	309.8 ± 21.6
12	324.0 ± 22.0	320.5 ± 23.5
16	344.6 ± 35.9	345.1 ± 25.0
Actual 20	395.3 ± 44.2	400.8 ± 36.8
Corrected 20	314.0 ± 26.8	318.4 ± 33.8
20 - 0 Weight gain†	33.0 ± 22.8	40.5 ± 19.6

*Mean ± S.D. in gm of pregnant animals.
†Group mean of [Corrected Day 20 weight - Day 0 weight].

Table 2

Cumulative Maternal Clinical Signs

Observation	Positive Control	Negative Control
Pretreatment (Days 0-5)		
Slight weight loss	1	1
Treatment (Days 6-15)		
Blood		
mouth/nose	1	1
vaginal area	1	
Foaming at mouth during misdosing		1
Inactive	1	
Red stained nose		1
Wheezing		2
Posttreatment and at Cesarean (Days 16-20)		
Uterus:		
enlarged, fluid filled		1
blood filled	1	
Rough hair coat, swollen hind leg	1	

Table 3

Summary of Maternal Data

	Positive Control	Negative Control
Number of animals assigned	25	25
Number of animals died	0	0
Percent of animals died	0	0
Number of animals pregnant	24	17
Percent of animals pregnant	96	68

Table 4

Mean* Uterine and Litter Data

	Positive Control	Negative Control
Number of litters	24	17
Mean values per litter		
Number of implantations	14.4 ± 4.9	14.1 ± 4.9
Number of resorptions	1.4 ± 1.9	1.5 ± 1.5
Percent resorptions†	10.0 ±14.9	10.8 ±12.0
Number of dead fetuses	0.1 ± 0.3	0
Percent dead fetuses§	0.8 ± 2.1	0
Number of live fetuses	12.8 ± 5.0	12.6 ± 5.1
Percent live fetuses‡	89.2 ±14.7	89.2 ±12.0
Live fetuses:		
Body weight (g)	3.5 ± 0.9**	4.3 ± 1.0
Body weight male fetuses (g)	3.6 ± 1.0**	4.4 ± 1.0
Body weight female fetuses (g)	3.4 ± 0.9	4.0 ± 0.8
Number of male fetuses	5.8 ± 2.7	6.9 ± 3.2
Percent male fetuses	42.5 ±18.9**	57.5 ±17.8

*Group mean ± S.D.
†Group mean of [resorptions per litter/implantations per litter] x 100
§Group mean of [dead fetuses per litter/implantations per litter] x 100
‡Group mean of [live fetuses per litter/implantations per litter] x 100
**Significantly different from the negative control.

Table 5

Description and Incidence of External Examination Findings

	Positive Control		Negative Control	
	No.	%	No.	%
No. of fetuses examined	308		215	
Anomalies				
Exencephaly	219	71		
Hydrocephaly	123	40		
Domed cranium	98	32	1	0
Flat cranium	62	20		
Pointed cranium	2	1		
Cleft palate	2	1		
Prognathism	9	3		
Brachygnathia	3	1		
Protruding tongue	17	6	1	0
Round body shape	5	2		
Short, curved body shape	1	0		
Spina bifida	1	0		
Umbilical hernia	1	0		
Short forelimb	1	0		
Short paw digits	1	0		
Forepaw digits long and separate	116	38		
Forepaw one digit long, others short	20	6		
Syndactyly	152	49		
Polydactyly	3	1		
Talipes equinovarus	276	90		
Short or curly tail	257	83		
Absence of tail	31	10		
Absence of anus	20	6		
Variants				
Anasarca	3	1		
Decreased definition of paws	35	11		
Underdeveloped, smooth skin	1	0		
Hemorrhage on cranium	9	3		

Table 6

Description and Incidence of Visceral Examination Findings

	Positive Control		Negative Control	
	No.	%	No.	%
No. of fetuses examined	148		105	
Anomalies				
Exencephaly	70	47		
Hydrocephaly	39	26		
Hydranencephaly	2	1		
Compressed or cystic brain	76	51		
Hypoplasia of olfactory bulb	49	33		
Meningocele	9	6		
Cystic spinal cord	1	1		
Spina bifida	1	1		
Open eye	1	1		
Brachygnathia	27	18		
Prognathism	1	1		
Agnathia	1	1		
Lobed tongue	1	1		
Protruding tongue	10	7		
Cleft palate	14	9		
Cleft nose and palate	1	1		
Abnormal turbinates	1	1		
Abnormal ventricle of heart	1	1		
Cystic kidney	3	2		
Absence of kidney	1	1		
Ectopic adrenal	2	1		
Absence of adrenal	1	1		
Ectopic testes*	2	3		
Ectopic ovary†	4	5		
Diaphragmatic hernia	1	1		

*Percent calculated with number of male fetuses - 69.
†Percent calculated with number of female fetuses - 79.

Table 6 (Continued)

Description and Incidence of Visceral Examination Findings

	Positive Control		Negative Control	
	No.	%	No.	%
No. of fetuses examined	148		105	
Variants				
Dilated ventricles of brain	112	76	1	1
Cerebral hemorrhage			3	3
Dilated arachnoid space	83	56		
Dilated foramen magnum	16	11		
Microphthalmia	3	2		
Small lens			1	1
Dilated renal pelvis	90	61	11	11
Small kidney	13	9	6	6
Hypoplasia of adrenals	3	2		
Undescended testes*	18	26		
Hypoplastic ovary†	1	1		

*Percent calculated with number of male fetuses - 69.
†Percent calculated with number of female fetuses - 79.

Table 7

Description and Incidence of Skeletal Examination Findings

	Positive Control		Negative Control	
	No.	%	No.	%
No. of fetuses examined	158		110	
Anomalies				
Domed cranium	74	47	1	1
Flat cranium	49	31		
Short maxilla and long mandible	6	4	1	1
Short mandible	13	8		
Cleft palate	26	16		
Kyphosis	70	44		
Scoliosis	5	3		
Missing lumbar vertebrae	3	2		
Missing ribs	4	2		
Branched or fused ribs	42	27		
Curvature of clavicle	34	22		
Malformed scapula	4	2		
Malformed humerus	5	3		
Short humerus	1	1		
Short radius	9	6		
Absence of radius	4	3		
Short ulna	2	1		
Malformed ulna	4	2		
Absence of ulna	1	1		
Fused metacarpals	1	1		
Short femur	1	1		
Short tibia	31	20		
Absence of tibia	2	1		
Short fibula	3	2		
Absence of fibula	1	1		
Tibia and fibula parallel	1	1		

Table 7 (Continued)

Description and Incidence of Skeletal Examination Findings

	Positive Control		Negative Control	
	No.	%	No.	%
No. of fetuses examined	158		110	
Variants				
Incomplete ossification				
Cranium	151	96	14	13
Vertebral centra	151	96	7	6
Zygomatic arch	5	3		
Pelvis	50	32		
Small or oval orbit	53	34		
Large orbit			1	1
Wavy ribs	47	30	2	2
Short ribs	29	18		
Lumbar rib	10	6		
Sternebrae				
Scrambled	7	4		
Fused	8	5		
Fewer than 3 ossified	6	4		

Table 8

Summary of Fetal Examination Findings

	Positive Control		Negative Control	
	No.	%	No.	%
External Exam				
Fetuses examined	308		215	
Litters examined	24		17	
Fetuses with anomalies	301*	98	1	0
Fetuses with variants	51*	17	0	0
Litters with anomalies	24*	100	1	6
Litters with variants	13*	54	0	0
Visceral Exam				
Fetuses examined	148		105	
Litters examined	23		16	
Fetuses with anomalies	120*	81	0	0
Fetuses with variants	144*	97	22	21
Litters with anomalies	23*	100	0	0
Litters with variants	23*	100	8	50
Skeletal Exam				
Fetuses examined	158		110	
Litters examined	23		17	
Fetuses with anomalies	137*	87	1	1
Fetuses with variants	157*	99	21	19
Litters with anomalies	22*	96	1	6
Litters with variants	23*	100	9	53
Summary of External, Visceral, Skeletal				
Fetuses examined	308		215	
Litters examined	24		17	
Fetuses with any anomalies	305*	99	1	0
Fetuses with any variants	301*	98	43	20
Litters with any anomalies	24*	100	1	6
Litters with any variants	24*	100	11	65

*Significantly different from the negative control.

Table 9

Summary of Ossification Data*

	Positive Control	Negative Control
Sternebrae	5.05 ± 0.98	5.40 ± 0.49
Caudal vertebrae	1.85 ± 2.13†	3.66 ± 1.12
Metacarpals	4.95 ± 1.31†	6.60 ± 0.84
Metatarsals	4.82 ± 2.83†	8.08 ± 0.52

*Mean values calculated on a per litter basis.
†Significantly different from negative control.

DISCUSSION

Evidence for a teratogenic effect is considered to be a dose-related increase in frequency of major malformations in the test groups compared to the vehicle control group. Major malformations, such as cleft palate, protruding tongue, exencephaly, brachygnathia, prognathia, scoliosis, kyphosis, branched or fused ribs, syndactyly, hindlimb deformity, missing or malformed bones, short, curly or missing tail, missing anus, were considered anomalies. Minor variations in the number or degree of ossification of sternebrae, caudal vertebrae, metatarsals, metacarpals, skull bones, vertebral centra, dilated renal pelvis, dilated ventricles of the brain, cerebral hemorrhage, wavy, short, and lumbar ribs were considered to be variants as they do not represent a specific malformation but a transient phase in development. However, if results show that variants occur at significantly higher frequency in the test groups than in the vehicle control group, this is evidence of embryotoxicity. Other manifestations of embryotoxicity are decreased body size and edema. Fetal deaths or resorptions are considered manifestations of maternal toxicity, not evidence of teratogenicity (12).

Aliverti et al proposed that delayed ossification provided a reliable index of retarded fetal development in teratogenic studies (13). In this study the number of sternebrae, caudal vertebrae, metatarsals, and metacarpals were counted in each fetus to determine if there were differences in development between the groups.

Results of the ETU dose group fetal examinations were similar to those reported by Khera (3) and Teramoto et al(4). Major malformations occurred in 98% of the fetuses and in all of the litters in the external examination. Major malformations seen frequently in this group were exencephaly, hydrocephaly, domed or flattened cranium, syndactyly, forepaw digits long and separate in appearance or one digit long and the others short, talipes equinovarus, and missing anus. Retarded development was evidenced in some fetuses by decreased definition of paws and low body weight.

Major malformations in the positive control Bouin's processed fetuses included exencephaly, hydrocephaly, compressed or cystic brain, hypoplasia of the olfactory bulb, brachygnathia, protruding tongue, and cleft palate. Variants seen in high frequency included dilated lateral, 3rd and 4th ventricles of brain, dilated renal pelvis, small kidney, and undescended testes. The spinal cord of many fetuses in this group was smaller than the vertebral canal creating an enlarged arachnoid space and dilated foramen magnum.

Major skeletal malformations found in high frequency in the positive control fetuses included abnormally shaped craniums, cleft palate, branched or fused ribs, missing, short, or misshaped bones of

the limbs. An abnormal body shape, termed "kyphosis" in the observations, was characterized by the head set forward on the neck, the mandible almost touching the chest, and vertebral column humpbacked in the thoracic region. Curvature of the clavicle was characterized by clavicle curving inward sharply giving a sunken chest appearance. Skeletal variants observed were delayed ossification of the cranium to include the frontal, parietal, interparietal and supraoccipital bones; delayed ossification of the pelvis to include short or unossified pubis or ischium; vertebral centra split, dumbbell-shaped or unossified; wavy ribs; short 13th rib; supernumerary lumbar rib; and small or oval orbit.

One negative control fetus had anomalies of the head characterized by domed shaped cranium, protruding tongue and short maxilla with long mandible which represents a 0.47% anomaly rate for fetuses in that group. Palmer (5) reported an incidence of spontaneous malformations in the rat of 0.41% major malformations from 51,349 control fetuses.

CONCLUSION

When given in daily oral doses of 40 mg/kg/day from Days 6 through 15 of gestation, the positive control substance, ethylene thiourea (ETU), produced teratogenic and embryotoxic effects in Sprague-Dawley rats. The LAIR teratology testing procedure for the Sprague-Dawley rat is a valid model for testing substances for teratogenic potential.

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Negative Control Substances

Chemical Name: Polysorbate 80 (Tween 80)

Chemical Abstract Service Registry No: 9005-65-6

Source: Fisher Scientific Co.
Fairlawn, New Jersey 07410

Lot No: 713137

Chemical Name: Ethanol, anhydrous

Chemical Abstract Service Registry No: 64-17-5

Phase I

Source: U.S. Industrial Chemicals
Tuscola, Illinois 61953

Lot No: 136

Phase II

Source: Aaper Alcohol and Chemical Co.
Louisville, Kentucky 40214

Lot No: DSP-KY-73

Chemical Name: Citric acid, monohydrate

Chemical Abstract Service Registry No: 77-92-9

Chemical Name: Sodium citrate

Chemical Abstract Service Registry No: None

Historical Listing of Major Study Events

29 Jun 82	Date of protocol request.
31 Aug 82	41 male rats arrived at LAIR for Phase I.
5 Oct 82	65 females arrived at LAIR for Phase I.
12 - 15 Oct 82	Phase I breeding.
19 - 30 Oct 82	Sperm-positive females dosed.
2 - 4 Nov 82	Cesarean sections on sperm-positive females.
13 Dec 82	52 males arrived at LAIR for Phase II.
20 Jan 83	103 females arrived at LAIR for Phase II.
7 - 12 Feb 83	Phase II breeding.
14 - 27 Feb 83	Sperm-positive females dosed.
28 Feb - 4 Mar 83	Cesarean sections on sperm-positive females.

Individual Maternal Body Weights (Grams) - Positive Control

Maternal ID	Day 0 Age	Date of Breeding	Date of Sacrifice	Pregnant	Day of Gestation 0	6	12	16	Actual 20	Correct 20	Weight Change
82D00683	(132)	15 Oct 82	04 Nov 82	No	(294)	(324)	(323)	(302)	(300)	(300)	(6)
82D00687	130	13 Oct 82	02 Nov 82	Yes	263	288	324	351	435	324	61
82D00701	130	13 Oct 82	02 Nov 82	Yes	281	308	262	209	279	271	-10
82D00707	130	13 Oct 82	02 Nov 82	Yes	280	311	332	355	410	331	51
82D00718	131	14 Oct 82	03 Nov 82	Yes	287	318	303	315	340	309	22
82D00720	131	14 Oct 82	03 Nov 82	Yes	292	326	329	335	405	316	24
82D00721	130	13 Oct 82	02 Nov 82	Yes	304	331	347	360	410	359	55
82D00725	130	13 Oct 82	02 Nov 82	Yes	282	316	329	372	444	325	43
82D00736	130	13 Oct 82	02 Nov 82	Yes	272	302	319	351	399	300	28
82D00754	130	13 Oct 82	02 Nov 82	Yes	299	331	360	398	475	350	51
83D00002	99	10 Feb 83	02 Mar 83	Yes	257	286	292	315	340	266	9
83D00006	97	08 Feb 83	28 Feb 83	Yes	286	322	320	323	403	319	33
83D00007	98	09 Feb 83	01 Mar 83	Yes	278	313	319	346	394	299	21
83D00008	99	10 Feb 83	02 Mar 83	Yes	257	279	305	336	371	286	29
83D00017	97	08 Feb 83	28 Feb 83	Yes	278	299	313	346	413	331	53
83D00027	98	09 Feb 83	01 Mar 83	Yes	279	314	317	353	389	312	33
83D00028	97	08 Feb 83	28 Feb 83	Yes	266	328	325	364	432	331	65
83D00036	99	10 Feb 83	02 Mar 83	Yes	290	326	363	388	414	351	61
83D00045	98	09 Feb 83	01 Mar 83	Yes	299	333	336	333	335	323	24
83D00064	97	08 Feb 83	28 Feb 83	Yes	280	302	314	350	431	325	45
83D00066	98	09 Feb 83	01 Mar 83	Yes	275	308	313	340	393	300	25
83D00081	100	11 Feb 83	03 Mar 83	Yes	263	315	336	369	358	269	6
83D00083	101	12 Feb 83	04 Mar 83	Yes	314	341	358	*	409	335	21
83D00084	100	11 Feb 83	03 Mar 83	Yes	272	321	333	355	455	335	63
83D00103	100	11 Feb 83	03 Mar 83	Yes	289	345	327	361	353	269	-20

* Weight not obtained.

Individual Maternal Body Weights (Grams) - Negative Control

Maternal ID	Day 0 Age	Date of Breeding	Date of Sacrifice	Pregnant	Day of Gestation 0	6	12	16	20 Actual	20 Correct	Weight Change
82D00682	130	13 Oct 82	02 Nov 82	Yes	270	295	317	350	402	317	47
82D00691	131	14 Oct 82	03 Nov 82	Yes	299	338	343	381	459	356	57
82D00694	(132)	15 Oct 82	04 Nov 82	No	(311)	(318)	(318)	(307)	(313)	(313)	(2)
82D00698	130	13 Oct 82	02 Nov 82	Yes	270	307	307	341	407	327	57
82D00703	130	13 Oct 82	02 Nov 82	Yes	267	306	335	328	343	327	60
82D00719	130	13 Oct 82	02 Nov 82	Yes	303	333	359	389	449	346	43
82D00724	130	13 Oct 82	02 Nov 82	Yes	286	319	302	333	365	311	25
82D00743	130	13 Oct 82	02 Nov 82	Yes	262	306	302	343	408	327	65
82D00749	(131)	14 Oct 82	03 Nov 82	No	(291)	(320)	(328)	(329)	(316)	(316)	(25)
82D00751	132	15 Oct 82	04 Nov 82	Yes	331	349	359	387	443	382	51
83D00001	98	09 Feb 83	01 Mar 83	Yes	263	287	303	313	340	279	16
83D00013	(97)	08 Feb 83	28 Feb 83	No	(277)	(278)	(289)	(296)	(302)	(302)	(25)
83D00019	98	09 Feb 83	01 Mar 83	Yes	272	316	322	357	395	278	6
83D00026	(97)	08 Feb 83	28 Feb 83	No	(314)	(331)	(325)	(314)	*	*	*
83D00039	97	08 Feb 83	28 Feb 83	Yes	296	329	337	350	402	353	57
83D00041	(98)	09 Feb 83	1 Mar 83	No	(282)	(324)	(317)	(313)	(300)	(300)	(18)
83D00049	(100)	11 Feb 83	03 Mar 83	No	(281)	(309)	(298)	(297)	(290)	(290)	(9)
83D00052	(97)	08 Feb 83	28 Feb 83	No	(285)	(314)	(317)	(328)	(332)	(332)	(47)
83D00062	99	10 Feb 83	02 Mar 83	Yes	265	282	302	334	373	273	8
83D00063	98	09 Feb 83	01 Mar 83	Yes	240	273	296	312	370	282	42
83D00070	100	11 Feb 83	03 Mar 83	Yes	265	294	311	343	385	285	20
83D00076	98	09 Feb 83	01 Mar 83	Yes	257	294	279	315	388	282	25
83D00082	101	12 Feb 83	04 Mar 83	Yes	301	335	351	*	451	356	55
83D00087	(100)	11 Feb 83	03 Mar 83	No	(262)	(295)	(297)	(293)	(286)	(286)	(24)
83D00095	101	12 Feb 83	04 Mar 83	Yes	276	303	324	*	434	331	55

* Weight not obtained.

APPENDIX C-1

Maternal Clinical Signs - Positive Control

Maternal ID	Study Day	Date	Signs
82D00721	20	2 Nov 82	Left side of uterus filled with blood at sacrifice
83D00006	0	8 Feb 83	Slight weight loss
83D00064	15	23 Feb 83	Blood in mouth
83D00066	8	17 Feb 83	Inactive
83D00081	6	17 Feb 83	Bloody vaginal discharge
83D00103	20	3 Mar 83	Rough hair coat, swollen right hind leg

Maternal Clinical Signs - Negative Control

Maternal ID	Study Day	Date	Signs
82D00691	14	28 Oct 82	Brief bleeding from mouth on introduction of gavage needle, not dosed
82D00703	20	2 Nov 82	Enlarged uterus, left horn filled with fluid at sacrifice
83D00039	15	23 Feb 83	Wheezing
83D00062	11	21 Feb 83	Misdose, slight foaming at mouth
83D00063	7	16 Feb 83	Red stained nose
	14	23 Feb 83	Wheezing
83D00082	0	12 Feb 83	Slight weight loss

Individual Uterine and Litter Data - Positive Control

Maternal ID	Implantations	Resorptions	Resorp.%	Number of Fetuses			
				Dead	Dead %	Live	Live %
[illegible]697	14	0	0	0	0	14	100
82D00701	1	0	0	0	0	1	100
82D00707	16	3	19	0	0	13	81
82D00718	7	4	57	0	0	3	43
82D00720	16	2	12	0	0	14	87
82D00721	16	8	50	0	0	8	50
82D00725	15	0	0	1	7	14	93
82D00736	14	2	14	0	0	12	86
82D00754	16	1	6	1	6	14	88
83D00002	18	3	17	0	0	15	83
83D00006	17	2	12	0	0	15	88
83D00007	17	2	12	0	0	15	88
83D00008	18	1	6	1	6	16	89
83D00017	16	1	6	0	0	15	94
83D00027	16	1	6	0	0	15	94
83D00028	17	0	0	0	0	17	100
83D00036	10	0	0	0	0	10	100
83D00045	1	0	0	0	0	1	100
83D00064	18	0	0	0	0	18	100
83D00066	18	3	17	0	0	15	83
83D00081	18	0	0	0	0	18	100
83D00083	13	0	0	0	0	13	100
83D00084	16	1	6	0	0	15	94
83D00103	17	0	0	0	0	17	100

Individual Uterine and Litter Data - Negative Control

Maternal ID	Implantations	Resorptions	Resorp.	Number of Fetuses Dead	Dead %	Live	Live %
82D00682	14	5	[illegible]	0	0	9	64
82D00691	18	1	[illegible]	0	0	17	94
82D00698	14	0	[illegible]	0	0	14	100
82D00703	1	0	[illegible]	0	0	1	100
82D00719	16	1	6	0	0	15	94
82D00724	9	3	33	0	0	6	67
82D00743	15	3	20	0	0	12	80
82D00751	10	1	10	0	0	9	90
83D00001	13	4	31	0	0	9	69
83D00019	18	0	[illegible]	0	0	18	100
83D00039	7	0	[illegible]	0	0	7	100
83D00062	19	0	[illegible]	0	0	19	100
83D00063	15	1	7	0	0	14	93
83D00070	19	1	5	0	0	18	95
83D00076	18	1	[illegible]	0	0	17	94
83D00082	16	2	12	0	0	14	88
83D00098	18	2	11	0	0	16	89

Fetal Sex and Weight - Positive Control

Maternal ID	Sex			Mean Weight(G) ± S.D.		
	Males	Females	Males(%)	Fetal	Males	Females
82D00687	7	7	50	4.68±.20	4.79±.20	4.57±.14
82D00701	0	1	0	2.30		2.30
82D00707	8	5	62	3.23±.25	3.31±.20	3.10±.29
82D00718	1	2	33	4.73±1.1	5.40	4.40±1.3
82D00720	7	7	50	3.41±.20	3.49±.22	3.34±.16
82D00721	6	2	75	2.99±.42	2.97±.48	3.05±.21
82D00725	7	7	50	4.71±.53	4.77±.58	4.64±.52
82D00736	3	9	25	5.03±.21	5.20±.17	4.98±.20
82D00754	7	7	50	5.62±.35	5.71±.28	5.53±.40
83D00002	5	10	33	2.60±.29	2.56±.23	2.63±.33
83D00006	10	5	67	3.02±.14	3.04±.16	2.98±.08
83D00007	6	9	40	3.53±.33	3.55±.52	3.52±.40
83D00008	5	11	31	2.71±.26	2.92±.29	2.62±.19
83D00017	8	7	53	2.81±.34	2.82±.44	2.80±.22
83D00027	6	9	40	2.71±.19	2.77±.15	2.68±.22
83D00028	5	12	29	2.92±.25	3.00±.19	2.88±.27
83D00036	7	3	70	3.31±.16	3.33±.14	3.27±.23
83D00045	0	1	0	3.60		3.60
83D00064	7	11	39	3.21±.14	3.31±.11	3.15±.12
83D00066	9	6	60	3.26±.26	3.36±.20	3.12±.29
83D00081	8	10	44	2.40±.16	2.41±.22	2.39±.11
83D00083	5	8	38	2.98±.30	3.26±.18	2.81±.22
83D00084	4	11	27	4.68±.28	4.68±.39	4.68±.24
83D00103	9	8	53	2.58±.19	2.66±.21	2.50±.12

Fetal Sex and Weight - Negative Control

Maternal ID	Sex: Males	Sex: Females	Sex: Males(%)	Mean Weight(G) ± S.D.: Fetal	Mean Weight(G) ± S.D.: Males	Mean Weight(G) ± S.D.: Females
82D00682	7	2	78	6.16±.26	6.21±.27	5.95±.07
82D00691	9	8	53	3.66±.57	3.98±.11	3.31±.68
82D00698	6	8	43	3.55±.25	3.77±.12	3.39±.19
82D00703	1	0	100	6.40	6.40	
82D00719	11	4	73	4.47±.35	4.57±.20	4.18±.53
82D00724	4	2	67	6.27±.29	6.40±.26	6.00±.14
82D00743	6	6	50	4.18±.28	4.27±.27	4.08±.28
82D00751	2	7	22	3.79±.33	4.20±.00	3.67±.27
83D00001	4	5	44	4.16±.14	4.20±.16	4.12±.13
83D00019	11	7	61	4.09±.22	4.20±.20	3.93±.14
83D00039	5	2	71	4.19±.27	4.18±.33	4.20±.14
83D00062	12	7	63	3.22±.27	3.25±.22	3.16±.36
83D00063	7	7	50	3.83±.50	3.96±.54	3.70±.46
83D00070	8	10	44	3.57±.20	3.70±.22	3.47±.12
83D00076	11	6	65	3.88±.19	3.96±.16	3.73±.15
83D00082	7	7	50	3.74±.44	3.96±.11	3.51±.54
83D00096	7	9	44	3.49±.15	3.56±.10	3.43±.17

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	Variants			Anomalies	
	No.*	No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
82D00687	14	6	6 Decreased definition paws	14	14 Exencephaly 14 Talipes equinovarus 2 Round body shape 11 Short or curly tail 1 Absence of tail
82D00701	1	1	1 Anasarca	1	1 Exencephaly 1 Syndactyly 1 Absence of tail 1 Absence of anus
82D00707	13	5	5 Decreased definition paws	13	13 Exencephaly 1 Pointed cranium 3 Round body shape 1 Syndactyly 13 Talipes equinovarus 8 Short or curly tail 5 Absence of tail 1 Absence of anus

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	Variants No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
82D00718	3	1	1 Anasarca 1 Decreased definition paws	3	3 Exencephaly 1 Flat cranium 1 Protruding tongue 1 Short forelimb 1 Syndactyly 3 Talipes equinovarus 2 Absence of tail 1 Absence of anus
82D00720	14	2	2 Decreased definition paws	14	7 Exencephaly 1 Flat cranium 1 Hydrocephaly 2 Talipes equinovarus 14 Short or curly tail 1 Absence of tail

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	No.*	Variants		Anomalies	
		No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
82D00721	8	2	1 Underdeveloped, smooth skin 1 Decreased definition paws	8	7 Exencephaly 3 Hydrocephaly 3 Flat cranium 1 Pointed cranium 4 Syndactyly 1 Polydactyly 8 Talipes equinovarus 5 Short or curly tail 3 Absence of tail
82D00725	14	0		14	13 Exencephaly 1 Hydrocephaly 3 Flat cranium 2 Cleft palate 3 Brachygnathia 1 Spina bifida 3 Syndactyly 12 Talipes equinovarus 10 Short or curly tail

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	Variants No.*	Variants No.+	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
82D00736	12	0		12	10 Exencephaly 6 Flat cranium 1 Short, curved body shape 8 Syndactyly 4 Forepaw digits long and separate 11 Talipes equinovarus 7 Short or curly tail 3 Absence of tail
82D00754	14	3	3 Hemorrhage on cranium	14	4 Exencephaly 5 Hydrocephaly 1 Flat cranium 13 Talipes equinovarus 13 Short or curly tail

* Number of fetuses examined.
+ Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	Variants No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
83D00002	15	6	6 Decreased definition paws	15	15 Exencephaly 11 Flat cranium 2 Domed cranium 1 Protruding tongue 9 Prognathism 12 Syndactyly 1 Polydactyly 13 Talipes equinovarus 14 Short or curly tail
83D00006	15	0		15	6 Exencephaly 1 Hydrocephaly 13 Domed cranium 2 Flat cranium 1 Protruding tongue 9 Forepaw digits long and separate 6 Forepaw 1 digit long and others short 15 Syndactyly 15 Talipes equinovarus 15 Short or curly tail

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	Variants			Anomalies	
	No.*	No.+	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
83D00007	15	0		15	5 Exencephaly 9 Hydrocephaly 4 Flat cranium 5 Domed cranium 1 Syndactyly 1 Forepaw digits long and separate 15 Talipes equinovarus 15 Short or curly tail
83D00008	16	0		16	15 Exencephaly 7 Hydrocephaly 10 Flat cranium 5 Domed cranium 11 Forepaw digits long and separate 13 Syndactyly 1 Forepaw 1 digit long and others short 16 Talipes equinovarus 16 Short or curly tail

* Number of fetuses examined.
\+ Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	No.*	Variants		Anomalies	
		No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
83D00017	15	1	1 Anasarca	15	13 Exencephaly 8 Hydrocephaly 4 Flat cranium 11 Domed cranium 13 Syndactyly 1 Polydactyly 10 Forepaw digits long and separate 1 Forepaw 1 digit long and others short 15 Talipes equinovarus 15 Short or curly tail
83D00027	15	0		15	7 Exencephaly 10 Hydrocephaly 9 Domed cranium 10 Protruding tongue 5 Forepaw digits long and separate 9 Forepaw 1 digit long and others short 15 Syndactyly 15 Talipes equinovarus 15 Short or curly tail

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	No.*	Variants: No.+	Variants: No. and Description of Each Variant	Anomalies: No.§	Anomalies: No. and Description of Each Anomaly
83D00028	17	0		17	17 Exencephaly 2 Hydrocephaly 13 Flat cranium 16 Forepaw digits long and separate 11 Syndactyly 17 Talipes equinovarus 17 Short or curly tail
83D00036	10	0		10	9 Exencephaly 9 Hydrocephaly 1 Flat cranium 1 Domed cranium 6 Forepaw digits long and separate 2 Forepaw 1 digit long and others short 2 Syndactyly 10 Talipes equinovarus 10 Short or curly tail

* Number of fetuses examined.
\+ Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	No.*	Variants		Anomalies	
		No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
83D00045	1	0		1	1 Exencephaly 1 Hydrocephaly 1 Domed cranium 1 Forepaw digits long and separate 1 Syndactyly 1 Talipes equinovarus 1 Short or curly tail
83D00064	18	0		18	10 Exencephaly 11 Hydrocephaly 16 Domed cranium 1 Flat cranium 18 Forepaw digits long and separate 16 Syndactyly 18 Talipes equinovarus 18 Short or curly tail 1 Absence of anus

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
83D00066	15	3	3 Decreased definition paws	15	7 Exencephaly 12 Hydrocephaly 13 Domed cranium 1 Flat cranium 1 Umbilical hernia 14 Forepaw digits long and separate 1 Forepaw 1 digit long and others short 1 Hindpaw short digits 8 Syndactyly 15 Talipes equinovarus 14 Short or curly tail
83D00081	18	9	9 Decreased definition paws	18	18 Exencephaly 13 Hydrocephaly 11 Domed cranium 4 Protruding tongue 9 Forepaw digits long and separate 14 Syndactyly 18 Talipes equinovarus 17 Short or curly tail

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Positive Control

Maternal ID	No.*	Variants		Anomalies	
		No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
83D00083	13	6	6 Hemorrhage on cranium	6	2 Short or curly tail 4 Absence of tail 4 Absence of anus
83D00084	15	0		15	15 Exencephaly 15 Hydrocephaly 11 Domed cranium 1 Forepaw digits long and separate 6 Syndactyly 15 Talipes equinovarus 14 Short or curly tail
83D00103	17	2	2 Decreased definition paws	17	9 Exencephaly 17 Hydrocephaly 12 Forepaw digits long and separate 7 Syndactyly 17 Talipes equinovarus 6 Short or curly tail 11 Absent tail 12 Absent anus

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal External Examination Findings - Negative Control

Maternal ID	No.*	Variants		Anomalies	
		No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
82D00682	9	0		0	
82D00691	17	0		0	
82D00698	14	0		0	
82D00703	1	0		1	1 Domed cranium 1 Protruding tongue
82D00719	15	0		0	
82D00724	6	0		0	
82D00743	12	0		0	
82D00751	9	0		0	
83D00001	9	0		0	
83D00019	18	0		0	
83D00039	7	0		0	
83D00062	19	0		0	
83D00063	14	0		0	
83D00070	18	0		0	
83D00076	17	0		0	
83D00082	14	0		0	
83D00096	16	0		0	

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Visceral Examination Findings - Positive Control

Maternal ID	Variants			Anomalies	
	No.*	No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
82D00687	7	7	6 Dilated ventricles of brain 3 Dilated renal pelvis	7	7 Exencephaly 4 Compressed or cystic brain 2 Hypoplasia of olfactory bulb
82D00701	1	1	1 Small kidney 1 Microphthalmia	1	1 Exencephaly 1 Compressed or cystic brain 1 Cystic spinal cord 1 Open eye 1 Agnathia 1 Lobed tongue 1 Cleft palate
82D00707	6	6	5 Dilated ventricles of brain 4 Small kidney 6 Dilated renal pelvis 3 Undescended testes 5 Dilated arachnoid space	6	6 Exencephaly 6 Compressed or cystic brain 1 Diaphragmatic hernia 2 Cystic kidney 2 Ectopic testes
82D00718	1	1	1 Dilated ventricles of brain	1	1 Exencephaly 1 Compressed or cystic brain

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Visceral Examination Findings - Positive Control

Maternal ID	Variants			Anomalies	
	No.*	No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
82D00720	7	7	7 Dilated ventricles of brain 5 Dilated arachnoid space 5 Dilated renal pelvis	4	4 Exencephaly
82D00721	4	4	4 Dilated ventricles of brain 1 Dilated arachnoid space 4 Dilated renal pelvis 2 Undescended testes	4	4 Exencephaly 4 Compressed or cystic brain 1 Protruding tongue
82D00725	6	6	6 Dilated ventricles of brain 3 Dilated renal pelvis 1 Undescended testes 1 Small kidney	5	5 Exencephaly 1 Compressed or cystic brain 1 Spina bifida 1 Prognathism 1 Abnormal turbinates 1 Cleft nose and palate
82D00736	6	6	6 Dilated ventricles of brain 2 Dilated arachnoid space 6 Dilated renal pelvis	4	4 Exencephaly 1 Compressed or cystic brain 3 Hypoplasia of olfactory bulb

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Visceral Examination Findings - Positive Control

Maternal ID	Variants No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
82D00754	7	6	6 Dilated ventricles of brain 3 Dilated renal pelvis	4	2 Exencephaly 2 Hydranencehaly
83D00002	7	7	5 Dilated ventricles of brain 7 Dilated arachnoid space 1 Dilated foramen magnum 5 Dilated renal pelvis 1 Small kidney 3 *Undescended testes*	7	3 Exencephaly 2 Hydrocephaly 7 Compressed or cystic brain 2 Hypoplasia of olfactory bulb 3 Brachygnathia 1 Protruding tongue 5 Cleft palate 2 Cystic kidney
83D00006	7	7	6 Dilated ventricles of brain 5 Dilated arachnoid space 2 Dilated renal pelvis	4	1 Hydrocephaly 1 Compressed or cystic brain 4 Brachygnathia 3 Protruding tongue 1 Hypoplasia of olfactory bulb
83D00007	7	7	7 Dilated ventricles of brain 2 Dilated arachnoid space	3	1 Hydrocephaly 2 Hypoplasia of olfactory bulb

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Visceral Examination Findings - Positive Control

Maternal ID	Variants			Anomalies	
	No.*	No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
83D00008	8	8	3 Dilated ventricles of brain 3 Dilated arachnoid space 7 Dilated renal pelvis 1 Undescended testes	8	5 Compressed or cystic brain 8 Hypoplasia of olfactory bulb
83D00017	7	7	7 Dilated ventricles of brain 7 Dilated arachnoid space 7 Dilated renal pelvis 5 Dilated foramen magnum	7	5 Exencephaly 6 Hydrocephaly 7 Compressed or cystic brain 3 Meningocele 7 Brachygnathia 6 Hypoplasia of olfactory bulb 1 Cystic kidney 1 Ectopic ovary
83D00027	7	7	7 Dilated ventricles of brain 7 Dilated arachnoid space 6 Dilated foramen magnum 3 Undescended testes	7	7 Exencephaly 7 Hydrocephaly 7 Compressed or cystic brain 6 Meningocele 7 Brachygnathia 4 Protruding tongue 5 Cleft palate

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Visceral Examination Findings - Positive Control

Maternal ID	Variants			Anomalies	
	No.*	No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
83D00028	9	7	3 Dilated ventricles of brain 3 Dilated arachnoid space 5 Dilated renal pelvis	8	8 Exencephaly 2 Hydrocephaly 6 Compressed or cystic brain 4 Hypoplasia of olfactory bulb 2 Brachygnathia
83D00036	5	5	4 Dilated ventricles of brain 3 Dilated arachnoid space 1 Dilated renal pelvis 1 Undescended testes	4	4 Hydrocephaly 1 Hypoplasia of olfactory bulb
83D00064	9	8	7 Dilated ventricles of brain 7 Dilated arachnoid space 2 Micropthalmia 8 Dilated renal pelvis	4	4 Hydrocephaly 1 Brachygnathia
83D00066	7	7	7 Dilated ventricles of brain 6 Dilated arachnoid space 3 Dilated foramen magnum 2 Dilated renal pelvis	7	7 Hydrocephaly 4 Compressed or cystic brain 5 Hypoplasia of olfactory bulb 3 Brachygnathia

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Visceral Examination Findings - Positive Control

Maternal ID	Variants			Anomalies	
	No.*	No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
83D00081	9	9	4 Dilated ventricles of brain 8 Dilated renal pelvis	9	5 Exencephaly 3 Hydrocephaly 6 Compressed or cystic brain 1 Protruding tongue 3 Cleft palate 9 Hypoplasia of olfactory bulb 1 Abnormal ventricle of heart
83D00083	6	6	2 Dilated ventricles of brain 5 Dilated renal pelvis	2	2 Compressed or cystic brain
83D00084	7	7	2 Dilated ventricles of brain 1 Dilated foramen magnum 7 Dilated arachnoid space 4 Dilated renal pelvis 1 Small kidney 1 Undescended testes	7	5 Exencephaly 7 Compressed or cystic brain 6 Hypoplasia of olfactory bulb
83D00103	8	8	7 Dilated ventricles of brain 8 Dilated arachnoid space 3 Hypoplasia of adrenals 5 Dilated renal pelvis 5 Small kidneys 1 Hypoplastic ovary 3 Undescended testes	8	3 Exencephaly 2 Hydrocephaly 6 Compressed or cystic brain 1 Absence of adrenal 1 Absence of kidney 2 Ectopic adrenals 3 Ectopic ovaries

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Visceral Examination Findings - Negative Control

Maternal ID	Variants No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
82D00682	4	0		0	
82D00691	8	1	1 Dilated brain ventricles	0	
82D00698	7	0		0	
82D00719	7	0		0	
82D00724	3	0		0	
82D00743	6	2	1 Dilated renal pelvis 1 Small kidney	0	
82D00751	5	2	1 Dilated renal pelvis 1 Small lens	0	
83D00001	4	0		0	
83D00019	9	4	1 Cerebral hemorrhage 3 Dilated renal pelvis	0	
83D00039	3	0		0	
83D00062	10	5	5 Small kidney	0	
83D00063	7	0		0	
83D00070	9	1	1 Dilated renal pelvis	0	
83D00076	8	0		0	
83D00082	7	5	5 Dilated renal pelvis	0	
83D00096	8	2	2 Cerebral hemorrhage	0	

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Skeletal Examination Findings - Positive Control

Maternal ID	No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
82D00687	7	7	Incomplete ossification of: 7 Cranium 6 Vertebral centra 3 Wavy ribs 1 Short rib 1 Fused sternebrae	7	5 Domed cranium 2 Flat cranium 4 Branched or fused ribs 2 Missing rib
82D00707	7	7	Incomplete ossification of: 7 Cranium 7 Vertebral centra 3 Wavy ribs 2 Short rib 2 Fused sternebrae	7	5 Domed cranium 2 Flat cranium 6 Short maxilla, long mandible 7 Kyphosis 1 Scoliosis 4 Branched or fused ribs
82D00718	2	2	Incomplete ossification of: 2 Cranium 2 Vertebral centra 1 Small or oval orbit 1 Wavy ribs	2	1 Domed cranium 1 Flat cranium

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Skeletal Examination Findings - Positive Control

Maternal ID	No.*	Variants: No.†	Variants: No. and Description of Each Variant	Anomalies: No.§	Anomalies: No. and Description of Each Anomaly
82D007[illegible]	7	7	Incomplete ossification of: 7 Cranium 5 Vertebral centra 1 Short rib	4	3 Domed cranium 1 Flat cranium
82D00721	4	4	Incomplete ossification of: 4 Cranium 4 Vertebral centra 4 Wavy ribs 2 Short rib	4	2 Domed cranium 2 Flat cranium 3 Branched or fused ribs 3 Missing lumbar vertebrae
82D00725	8	8	Incomplete ossification of: 7 Cranium 7 Vertebral centra 1 Small or oval orbit 5 Wavy ribs 3 Fused sternebrae	8	4 Domed cranium 3 Flat cranium 2 Short mandible 2 Cleft palate 5 Branched or fused ribs 2 Missing ribs 1 Malformed humerus 1 Absence of radius 1 Short ulna 1 Absence of ulna 1 Short tibia 1 Short fibula

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Skeletal Examination Findings - Positive Control

Maternal ID	Variants No.*	Variants No.+	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
82D00736	6	6	Incomplete ossification of: 6 Cranium 6 Vertebral centra 4 Wavy ribs 1 Lumbar rib 1 Short rib	6	2 Domed cranium 3 Flat cranium 4 Branched or fused ribs 4 Kyphosis
82D00754	7	7	Incomplete ossification of: 7 Cranium 7 Vertebral centra 1 Lumbar rib	0	

* Number of fetuses examined.
\+ Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Skeletal Examination Findings - Positive Control

Maternal ID	No.*	Variants: No.†	Variants: No. and Description of Each Variant	Anomalies: No.§	Anomalies: No. and Description of Each Anomaly
83D00002	7‡	7	Incomplete ossification of: 7 Cranium 7 Vertebral centra 7 Pelvis 5 Small or oval orbit 6 Wavy ribs 2 Lumbar rib Sternebrae: 2 Scrambled 2 Fused	7	2 Domed cranium 5 Flat cranium 3 Short mandible 7 Cleft palate 5 Branched or fused ribs 4 Malformed scapula 3 Curvature of clavicle 6 Kyphosis 3 Scoliosis 4 Malformed humerus 4 Short radius 3 Absent radius 4 Malformed ulna 1 Absence of tibia 5 Short tibia 1 Absence of fibula

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.
‡One fetus damaged in processing not included.

Description and Incidence of Fetal Skeletal Examination Findings - Positive Control

Maternal ID	Variants			Anomalies	
	No.*	No.+	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
83D00006	8	8	Incomplete ossification of: 8 Cranium 8 Vertebral centra 1 Wavy ribs 2 Short rib 4 Lumbar rib 6 Small or oval orbit	8	7 Domed cranium 1 Flat cranium 2 Branched or fused ribs
83D00007	8	8	Incomplete ossification of: 8 Cranium 8 Vertebral centra 4 Pelvis 1 Small or oval orbit	5	4 Domed cranium 4 Kyphosis 1 Short tibia
83D00008	8	8	Incomplete ossification: 8 Cranium 8 Vertebral centra 6 Pelvis 8 Small or oval orbit 2 Wavy ribs	8	8 Flat cranium 7 Cleft palate 2 Branched or fused ribs 4 Short mandible 8 Kyphosis 8 Curvature of clavicle 3 Short tibia

* Number of fetuses examined.
+ Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Skeletal Examination Findings - Positive Control

Maternal ID	Variants No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
83D00017	8	8	Incomplete ossification: 8 Cranium 1 Zygomatic arch 8 Vertebral centra 8 Pelvis 5 Small or oval orbit 3 Wavy ribs Sternebrae: 4 Scrambled 1 Fewer than 3 ossified	8	5 Domed cranium 2 Flat cranium 2 Cleft palate 2 Branched or fused ribs 2 Curvature of clavicle 1 Short radius 1 Short humerus 1 Short ulna 1 Short femur 1 Absence of tibia 1 Short fibula 1 Tibia and fibula parallel
83D00027	7‡	7	Incomplete ossification: 7 Cranium 7 Vertebral centra 5 Pubis 4 Small or oval orbit	7	4 Domed cranium 2 Flat cranium 4 Cleft palate 4 Short mandible 5 Curvature of clavicle 4 Kyphosis 1 Short tibia

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.
‡One fetus damaged in processing not included.

Description and Incidence of Fetal Skeletal Examination Findings - Positive Control

Maternal ID	Variants No.*	Variants No.+	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
83D00028	8	8	Incomplete ossification: 8 Cranium 8 Vertebral centra 2 Pelvis 7 Small or oval orbit 1 Short rib	8	8 Flat cranium 1 Cleft palate 4 Kyphosis 2 Short radius 8 Short tibia 1 Fused metacarpals
83D00036	5	5	Incomplete ossification 5 Cranium 1 Zygomatic arch 5 Vertebral centra 1 Pelvis 1 Small or oval orbit 1 Short rib	5	2 Domed cranium 3 Flat cranium 2 Kyphosis 4 Curvature of clavicle
83D00045	1	1	Incomplete ossification: 1 Cranium 1 Vertebral centra 1 Small or oval orbit	1	1 Domed cranium

* Number of fetuses examined.
\+ Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Skeletal Examination Findings - Positive Control

Maternal ID	Variants No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
83D00064	9	9	Incomplete ossification: 9 Cranium 7 Vertebral centra 4 Small or oval orbit 1 Wavy ribs 2 Short rib	8	7 Domed cranium 1 Flat cranium 2 Kyphosis
83D00066	8	7	Incomplete ossification: 7 Cranium 7 Vertebral centra 3 Small or oval orbit 1 Wavy ribs	7	4 Domed cranium 3 Flat cranium 7 Kyphosis
83D00081	9	9	Incomplete ossification: 9 Cranium 9 Vertebral centra 9 Pelvis 4 Small or oval orbit 5 Wavy rib 1 Lumbar rib 6 Short rib 1 Scrambled sternebrae	9	7 Domed cranium 2 Flat cranium 3 Cleft palate 1 Branched or fused ribs 1 Scoliosis 8 Kyphosis 2 Short radius 9 Short tibia 1 Short fibula

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Skeletal Examination Findings - Positive Control

Maternal ID	Variants No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
83D00083	7	7	Incomplete ossification: 5 Cranium 7 Vertebral centra 1 Pelvis 1 Wavy ribs 2 Short rib	1	1 Branched or fused ribs
83D00084	8	8	Incomplete ossification: 5 Cranium 8 Vertebral centra 1 Lumbar rib 1 Short rib	8	8 Curvature of clavicle 7 Kyphosis
83D00103	9	9	Incomplete ossification: 9 Cranium 3 Zygomatic arch 9 Vertebral centra 7 Pelvis 2 Small or oval orbit 7 Wavy ribs 7 Short rib Sternebrae: 5 Fewer than 3 ossified	9	9 Domed cranium 9 Branched or fused ribs 7 Kyphosis 4 Curvature of clavicle 3 Short tibia

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Skeletal Examination Findings - Negative Control

Maternal ID	No.*	Variants		Anomalies	
		No.†	No. and Description of Each Variant	No.§	No. and Description of Each Anomaly
82D00682	5	0		0	
82D00691	9	3	Incomplete ossification: 3 Cranium 1 Wavy ribs	0	
82D00698	7	0		0	
82D00703	1	1	1 Large orbit	1	1 Domed cranium 1 Short maxilla/long mandible
82D00719	8	0		0	
82D00724	3	0		0	
82D00743	6	5	Incomplete ossification: 5 Cranium	0	
82D00751	4	0		0	
83D00001	5	2	Incomplete ossification: 2 Cranium 1 Wavy ribs	0	
83D00019	9	2	Incomplete ossification: 2 Vertebral centra	0	
83D00039	4	0		0	
83D00062	9	3	Incomplete ossification: 1 Cranium 3 Vertebral centra	0	

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Description and Incidence of Fetal Skeletal Examination Findings - Negative Control

Maternal ID	Variants No.*	Variants No.†	Variants No. and Description of Each Variant	Anomalies No.§	Anomalies No. and Description of Each Anomaly
83D00063	7	1	Incomplete ossification: 1 Cranium	0	
83D00070	9	1	Incomplete ossification: 1 Vertebral centra	0	
83D00076	9	0		0	
83D00082	7	0		0	
83D00096	8	3	Incomplete ossification: 2 Cranium 1 Vertebral centra	0	

* Number of fetuses examined.
† Number of fetuses with variants.
§ Number of fetuses with anomalies.

Incidence of External, Visceral, and Skeletal Examination Findings - Positive Control

Maternal ID	External					Visceral					Skeletal				
	Number Examined	Anomalies No.	%	Variants No.	%	Number Examined	Anomalies No.	%	Variants No.	%	Number Examined	Anomalies No.	%	Variants No.	%
82D00687	14	14	100	6	43	7	7	100	7	100	7	7	100	7	100
82D00701	1	1	100	1	100	1	1	100	1	100	-	-	-	-	-
82D00707	13	13	100	5	38	6	6	100	6	100	7	7	100	7	100
82D00718	3	3	100	1	33	1	1	100	1	100	2	2	100	2	100
82D00720	14	14	100	2	14	7	4	57	7	100	7	4	57	7	100
82D00721	8	8	100	2	25	4	4	100	4	100	4	4	100	4	100
82D00725	14	14	100	0	0	6	5	83	6	100	8	8	100	8	100
82D00736	12	12	100	0	0	6	4	67	6	100	6	6	100	6	100
82D00754	14	14	100	3	21	7	4	57	6	86	7	0	0	7	100
83D00002	15	15	100	6	40	7	7	100	7	100	7*	7	100	7	100
83D00006	15	15	100	0	0	7	4	57	7	100	8	8	100	8	100
83D00007	15	15	100	0	0	7	3	43	7	100	8	5	63	8	100
83D00008	16	16	100	0	0	8	8	100	8	100	8	8	100	8	100
83D00017	15	15	100	1	7	7	7	100	7	100	8	8	100	8	100
83D00027	15	15	100	0	0	7	7	100	7	100	7*	7	100	7	100
83D00028	17	17	100	0	0	9	8	89	7	78	8	8	100	8	100
83D00036	10	10	100	0	0	5	4	80	5	100	5	5	100	5	100
83D00045	1	1	100	0	0	-	-	-	-	-	1	1	100	1	100
83D00064	18	18	100	0	0	9	4	44	8	89	9	8	89	9	100
83D00066	15	15	100	3	20	7	7	100	7	100	8	7	88	7	88
83D00081	18	18	100	9	50	9	9	100	9	100	9	9	100	9	100
83D00083	13	6	46	6	46	6	2	33	6	100	7	1	14	7	100
83D00084	15	15	100	0	0	7	7	100	7	100	8	8	100	8	100
83D00103	17	17	100	2	12	8	8	100	8	100	9	9	100	9	100

* One fetus damaged in processing not included in skeletal examination.

Incidence of External, Visceral, and Skeletal Examination Findings - Negative Control

Maternal ID	External					Visceral					Skeletal				
	Number Examined	Anomalies No.	%	Variants No.	%	Number Examined	Anomalies No.	%	Variants No.	%	Number Examined	Anomalies No.	%	Variants No.	%
82D00682	9	0	0	0	0	4	0	0	0	0	5	0	0	0	0
82D00691	17	0	0	0	0	8	0	0	1	13	9	0	0	3	33
82D00698	14	0	0	0	0	7	0	0	0	0	7	0	0	0	0
82D00703	1	1	100	0	0	-	-	-	-	-	1	1	100	1	100
82D00719	15	0	0	0	0	7	0	0	0	0	8	0	0	0	0
82D00724	6	0	0	0	0	3	0	0	0	0	3	0	0	0	0
82D00743	12	0	0	0	0	6	0	0	2	33	6	0	0	5	83
82D00751	9	0	0	0	0	5	0	0	2	40	4	0	0	0	0
83D00001	9	0	0	0	0	4	0	0	0	0	5	0	0	2	40
83D00019	18	0	0	0	0	9	0	0	4	44	9	0	0	2	22
83D00039	7	0	0	0	0	3	0	0	0	0	4	0	0	0	0
83D00062	19	0	0	0	0	10	0	0	5	50	9	0	0	3	33
83D00063	14	0	0	0	0	7	0	0	0	0	7	0	0	1	14
83D00070	18	0	0	0	0	9	0	0	1	11	9	0	0	1	11
83D00076	17	0	0	0	0	8	0	0	0	0	9	0	0	0	0
83D00082	14	0	0	0	0	7	0	0	5	71	7	0	0	0	0
83D00096	16	0	0	0	0	8	0	0	2	25	8	0	0	3	38

Incidence of Anomalies and Variants
Positive Control

Maternal ID	Number Examined	Anomalies No.	Anomalies %	Variants No.	Variants %
82D00687	14	14	100	14	100
82D00701	1	1	100	1	100
82D00707	13	13	100	13	100
82D00718	3	3	100	3	100
82D00720	14	14	100	14	100
82D00721	8	8	100	8	100
82D00725	14	14	100	14	100
82D00736	12	12	100	12	100
82D00754	14	14	100	13	93
83D00002*	15	15	100	14	93
83D00006	15	15	100	15	100
83D00007	15	15	100	15	100
83D00008	16	16	100	16	100
83D00017	15	15	100	15	100
83D00027*	15	15	100	14	93
83D00028	17	17	100	15	88
83D00036	10	10	100	10	100
83D00045	1	1	100	1	100
83D00064	18	18	100	17	94
83D00066	15	15	100	14	93
83D00081	18	18	100	18	100
83D00083	13	9	69	13	100
83D00084	15	15	100	15	100
83D00103	17	17	100	17	100

*One fetus damaged in processing not included in skeletal examination.

Incidence of Anomalies and Variants
Negative Control

Maternal ID	Number Examined	Anomalies No.	%	Variants No.	%
82b00682	9	0	0	0	0
82b00691	17	0	0	4	24
82b00696	14	0	0	0	0
82b00703	1	1	100	1	100
82b00719	15	0	0	0	0
82b00723	6	0	0	0	0
82b00743	12	0	0	7	58
82b00751	9	0	0	2	22
83b00001	9	0	0	2	22
83b00019	18	0	0	6	33
83b00035	7	0	0	0	0
83b00062	19	0	0	8	42
83b00063	14	0	0	1	7
83b00070	18	0	0	2	11
83b00076	17	0	0	0	0
83b00082	14	0	0	5	36
83b00096	19	0	0	6	31

Fetal Ossification Data - Positive Control

Maternal ID	No. Fetuses	Mean Number Ossified			
		Sternebrae	Caudal Vertebrae	Metacarpals	Metatarsals
82D00687	7	5.71	2.43	6.00	8.57
82D00707	7	4.57	0	5.86	6.43
82D00718	2	6.00	3.00	6.00	7.50
82D00720	7	5.86	3.57	5.71	8.00
82D00721	4	5.25	0	5.75	3.75
82D00725	8	5.38	7.13	4.50	8.00
82D00736	6	6.00	5.50	6.00	1.67
82D00754	7	6.00	6.00	6.86	10.00
83D00002	7*	4.86	0	5.14	3.86
83D00006	8	5.88	1.88	3.00	0.86
83D00007	8	5.38	1.88	4.25	6.75
83D00008	8	4.63	0	3.38	1.63
83D00017	8	4.63	0.13	3.88	2.63
83D00027	7*	4.00	0.71	1.86	2.00
83D00028	8	5.38	0.63	5.88	2.25
83D00036	5	5.20	0.60	3.60	6.60
83D00045	1	5.00	3.00	6.00	6.00
83D00064	9	5.89	1.78	5.22	0.67
83D00066	8	4.75	0.63	4.38	2.25
83D00081	9	4.56	0.11	4.33	2.44
83D00083	7	3.43	0	5.71	6.86
83D00084	8	6.00	3.50	7.00	7.25
83D00103	9	1.89	0	3.56	4.89

* One fetus damaged in processing not included.

Fetal Ossification Data - Negative Control

Maternal ID	No. Fetuses	Mean Number Ossified			
		Sternebrae	Caudal Vertebrae	Metacarpals	Metatarsals
82D00685	7	6.00	5.29	8.00	8.00
82D00691	9	5.78	5.56	6.44	7.78
82D00698	7	5.57	2.86	6.00	7.43
82D00703	1	6.00	4.00	8.00	8.00
82D00719	8	6.00	5.00	8.00	8.25
82D00724	7	6.00	5.57	8.00	10.00
82D00743	6	6.00	3.17	6.00	8.00
82D00751	4	4.75	3.25	6.00	8.00
83D00001	5	5.40	4.00	6.00	8.00
83D00019	9	5.22	5.00	6.44	8.00
83D00059	4	5.00	2.25	6.50	8.00
83D00062	9	4.89	2.33	6.00	8.00
83D00063	7	5.43	3.14	6.86	8.00
83D00070	9	4.89	5.00	6.00	8.00
83D00076	8	5.11	5.00	6.00	8.00
83D00082	7	5.14	5.43	6.00	7.86
83D00096	8	4.63	3.25	6.00	8.00

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